

The Future of Forensic DNA

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National Institute of Standards and Technology

Gaithersburg, Maryland

United States of America



Checks and Controls on DNA Results



Community	FBI Quality Assurance Standards (<i>and interlaboratory studies</i>)
Laboratory	ASCLD/LAB Accreditation and Audits
Analyst	Proficiency Tests & Continuing Education
Method/Instrument	Validation of Performance <i>(along with traceable standard sample)</i>
Protocol	Standard Operating Procedure is followed
Data Sets	Allelic ladders, positive and negative amplification controls, and reagent blanks are used
Individual Sample	Internal size standard present in every sample
Interpretation of Result	Second review by qualified analyst/supervisor
Court Presentation of Evidence	Defense attorneys and experts with power of discovery requests

Presentation Outline

- **Introduction to NIST**

- Our role with forensic DNA in the United States
- Some current projects

- **Near-term future**

- New autosomal STR loci for expanded core loci
- Expanded use of databases (e.g., familial searching)
- Rapid DNA testing

- **More distant future**

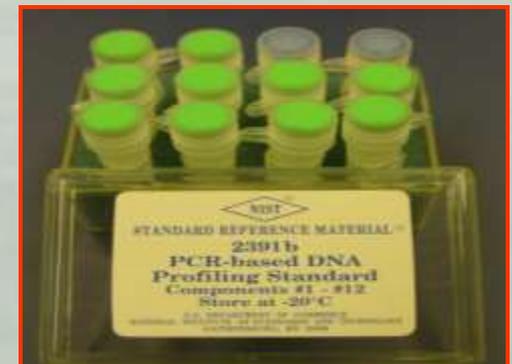
- Loci besides STRs for identity testing?
- Phenotyping capabilities?
- Next-generation DNA sequencing?

NIST History and Mission

- National Institute of Standards and Technology (NIST) was created in 1901 as the National Bureau of Standards (NBS). The name was changed to NIST in 1988.
- NIST is **part of the U.S. Department of Commerce** with a mission to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.
- NIST supplies over 1,300 Standard Reference Materials (SRMs) for industry, academia, and government **use in calibration of measurements.**
- **NIST defines time for the U.S.**

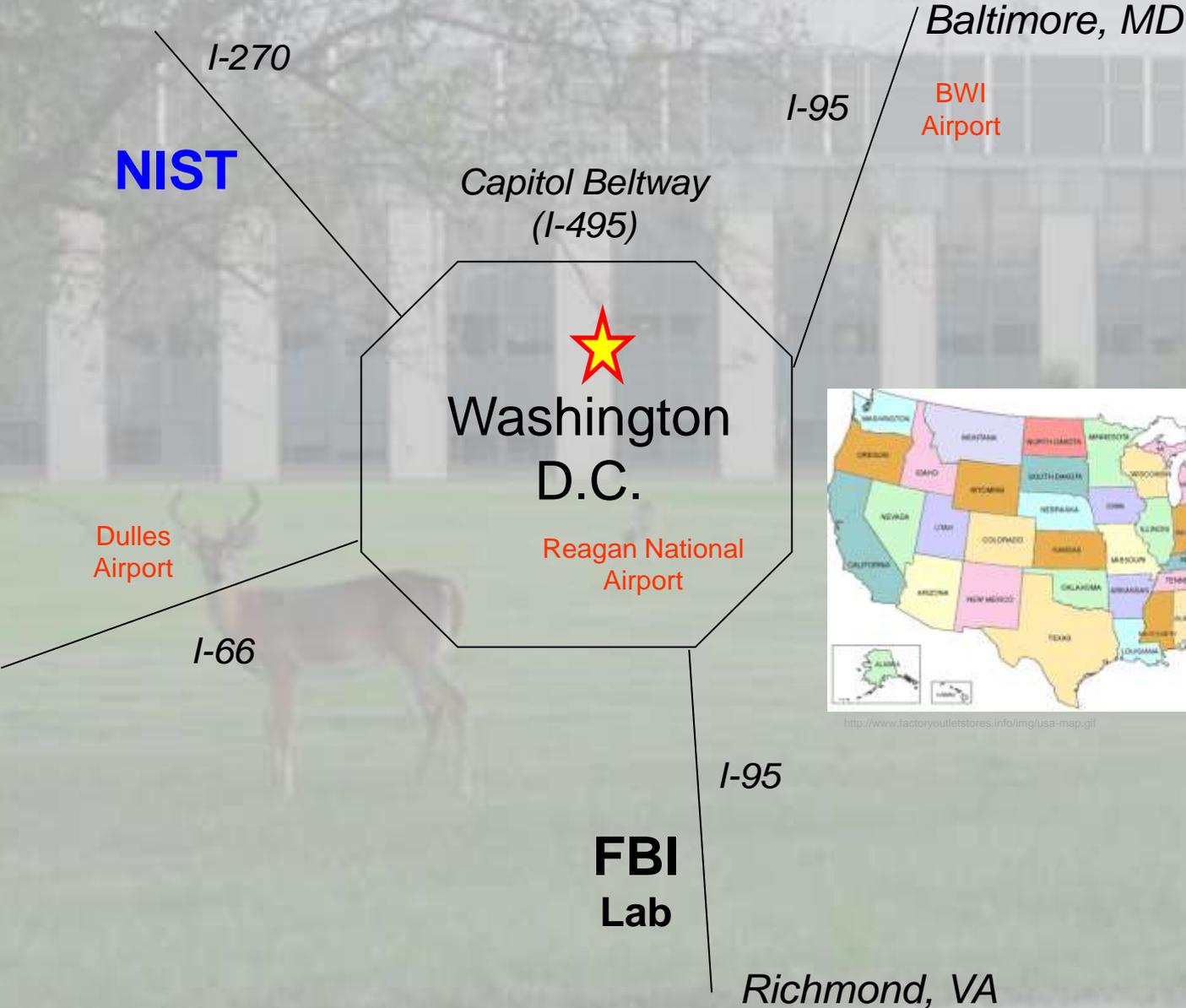


\$686 for 3 jars



DNA typing standard

Location of NIST



NIST Today

Major Assets

- ~ 2,900 employees
- ~ 2600 associates and facilities users
- ~ 400 NIST staff on about 1,000 national and international standards committees
- **4 Nobel Prizes in Physics in past 15 years**
(including 2012 to David Wineland for quantum physics)



Major Programs

- **NIST Laboratories**
- Baldrige National Quality Program
- Hollings Manufacturing Extension Partnership
- Technology Innovation Program

Joint NIST/University Institutes:

- JILA
- Joint Quantum Institute
- Institute for Bioscience & Biotechnology Research
- Hollings Marine Laboratory

Current Activities at NIST

Standard Reference Materials

- SRM 2372 (DNA quantitation standard)
- SRM 2391c (STR typing)

Technology Evaluation and Development

- Rapid multiplex PCR protocols (multiplex STR amplification in <35 min)
- Low-level DNA studies
- Mixture interpretation – research and training materials
- Unusual STR allele characterization
- New STR loci and assays (STR 26plex, kit concordance, InDels & SNPs)

• Training Materials

- Workshops on mixture interpretation and CE troubleshooting
- Third edition of *Forensic DNA Typing* textbook (2010, 2012, & 2014)

NIST Reference Materials for Forensic DNA Measurement Assurance



Margaret Kline



DNA quantity measurement calibration

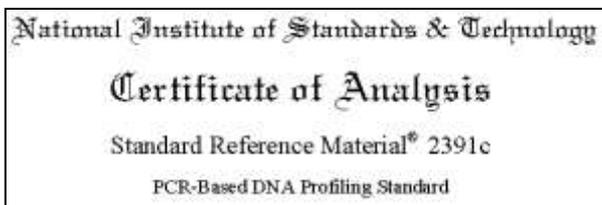


Autosomal and Y-chromosome short tandem repeat (STR) measurement calibration

Standard Reference Materials (SRMs)

<http://www.nist.gov/srm/>

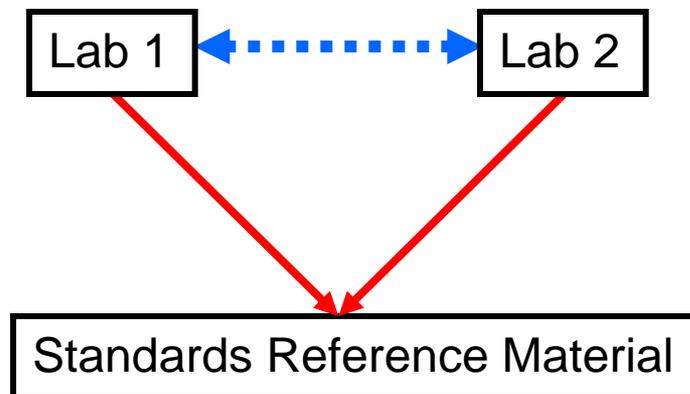
Traceable standards to ensure accurate measurements in crime laboratories



**Helps meet FBI QAS
and ISO 17025
requirements**



SRM 2391c – Autosomal and Y-STRs
SRM 2392-I – mtDNA
SRM 2372 – DNA quantitation



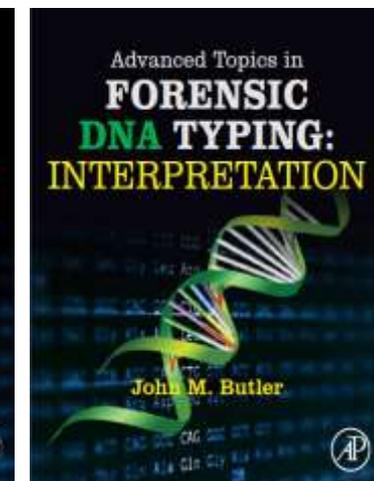
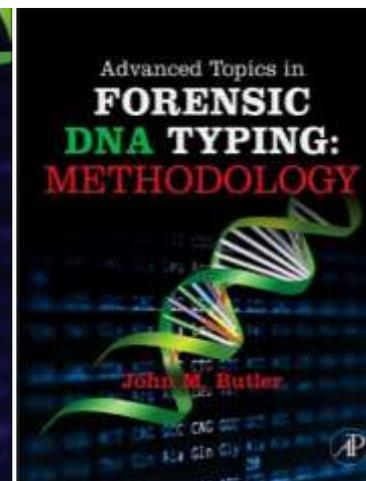
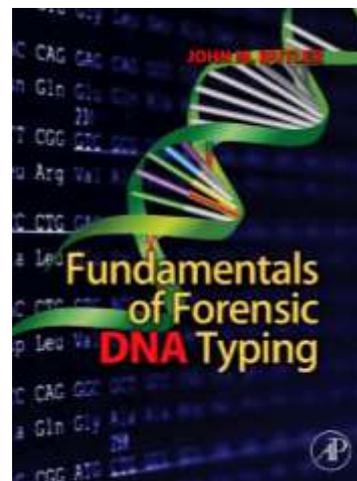
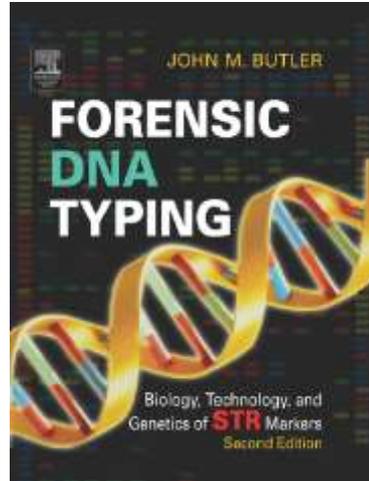
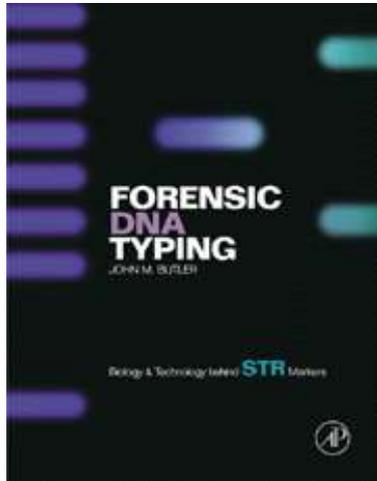
**Calibration with SRMs
enables confidence in
comparisons of results
between laboratories**

Forensic DNA Typing Textbooks Have Set the Standard for the Field

1st Edition

2nd Edition

3rd Edition (3 volumes)



Jan 2001

Feb 2005

Sept 2009

Aug 2011

Fall 2014

335 pages

688 pages

520 pages

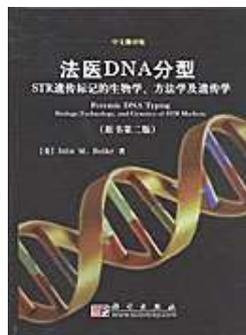
704 pages

(being written)

~500 pages

Language Editions

Chinese (2007)



Japanese (2009)



NIST **STRBase** Website

Serving the Forensic DNA Community for >15 Years



Short Tandem Repeat DNA Internet Database



NIST [Standard Reference Database](#) SRD 130

[\[Recent Updates\]](#)

Serving the forensic DNA and human identity testing communities for over 10 years... These data are intended to benefit research and application of short tandem repeat DNA markers to human identity testing. The authors are solely responsible for the information herein.

Please Rate Our Products and Services: <http://tsapps.nist.gov/MSDSurvey/default.aspx?ID=5&DB=130>

This database has been accessed **458551** times since 10/02/97. (Counter courtesy www.digits.com - see [disclaimer](#).)

Created by [John M. Butler](#)
and [Dennis J. Reeder](#) (*NIST Biochemical Science Division*),
with invaluable help from Jan Redman, Christian Ruitberg and Michael Tung
Site creators' curriculum vitae available using links above.

Partial support for the design and maintenance of this website is being provided by [The National Institute of Justice](#) through the [NIST Office of Law Enforcement Standards](#).

General Information

- [Purpose of STRBase/NAR 2001 Paper describing STRBase/Overview Presentation](#)
- [Publications and Presentations from NIST Human Identity Project Team](#) ◆
- [NIJ-Funded Projects](#) ◆
- [Training Materials](#) ◆
- [Links to other web sites](#) ◆
- [Glossary of commonly used terms](#)

<http://www.cstl.nist.gov/strbase/>

Publications on Forensic DNA

from the NIST Applied Genetics Group

- **144 publications since 2002**
 - 40 in the past 2 years
 - Includes journal articles, book chapters, and textbooks
- References are all listed on STRBase
 - <http://www.cstl.nist.gov/strbase/NISTpub.htm>
 - Many are available directly from STRBase



Most of our articles are published in *Forensic Sci. Int. Genetics* – currently the highest impact journal in the field

136 page report written by Kevin Kiesler

NIST Report to the FBI:
Plex-ID Electrospray Time-of-Flight Mass
Spectrometer for Mitochondrial DNA
Base Composition Profiling

Experiments performed and report written by: Kevin Kiesler, M.S. (NIST)

Under the direction of: Dr. Peter Vallone (NIST)

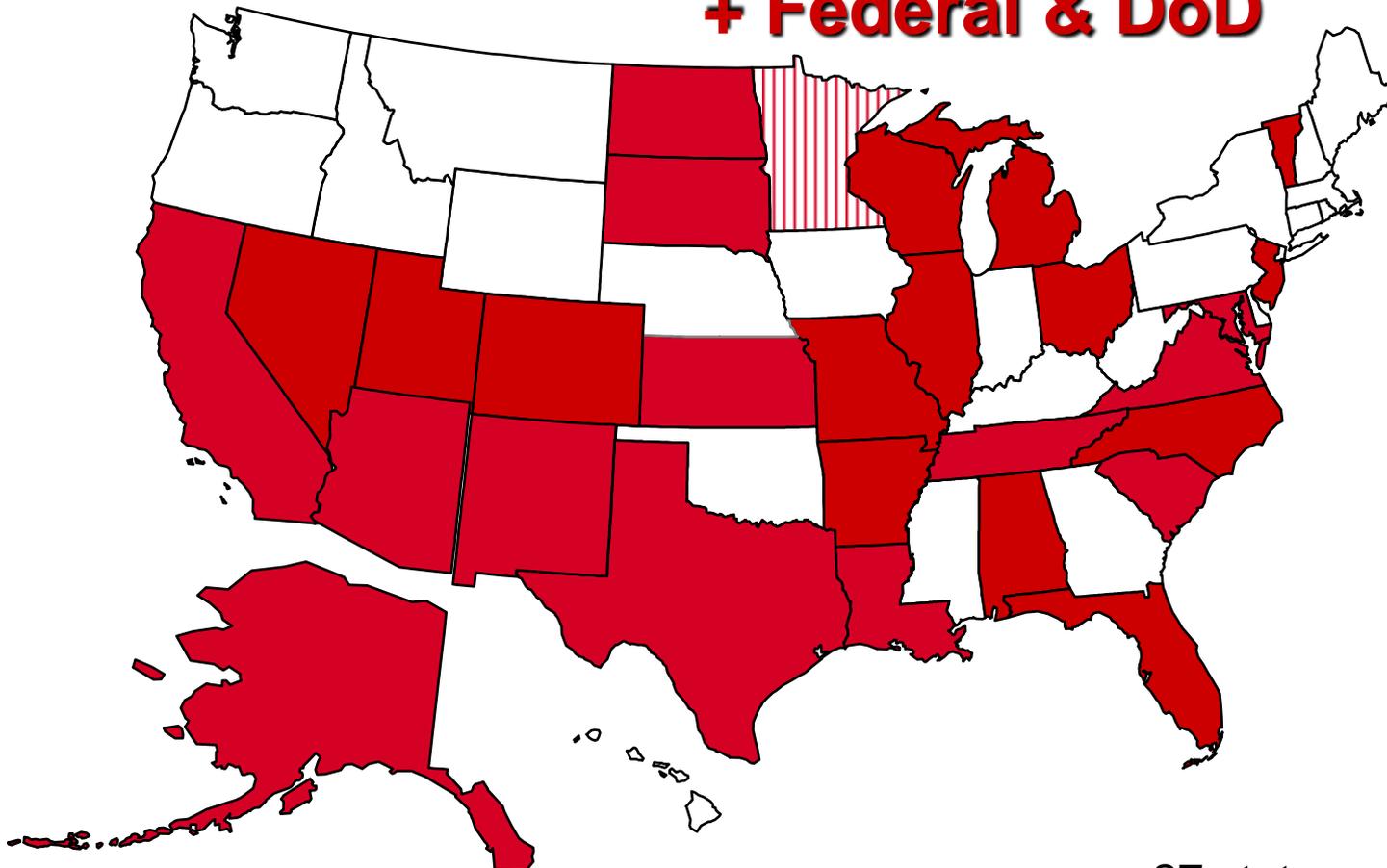
Stages of Forensic DNA Progression

Stages	Time Frame	Description
Exploration	1985-1995	Beginnings, different methods tried (RFLP and early PCR)
Stabilization	1995-2005	Standardization to STRs, selection of core loci, implementation of Quality Assurance Standards
Growth	2005-2013	Rapid growth of DNA databases, extended applications pursued
<i>Sophistication</i>	<i>The Future</i>	<i>Expanding tools available, confronting privacy concerns</i>

More than Half of the U.S. Permits Arrestee DNA Testing

U.S. Supreme Court case *Maryland v King*
(5-4 ruling June 3, 2013)

+ Federal & DoD



Alabama
Alaska
Arizona
Arkansas
California
Colorado
Florida
Georgia
Illinois
Indiana
Iowa
Kansas
Kentucky
Louisiana
Maryland
Massachusetts
Michigan
Minnesota
Missouri
Montana
Nebraska
Nevada
New Jersey
New Mexico
New York
North Carolina
North Dakota
Ohio
Oklahoma
Oregon
Pennsylvania
Rhode Island
South Carolina
South Dakota
Tennessee
Texas
Utah
Virginia
Washington
West Virginia
Wisconsin
Wyoming

27 states as of Sept 2013



KNOW THE CASES : UNDERSTAND THE CAUSES : FIX THE SYSTEM

ABOUT : DONATE : NEWS &



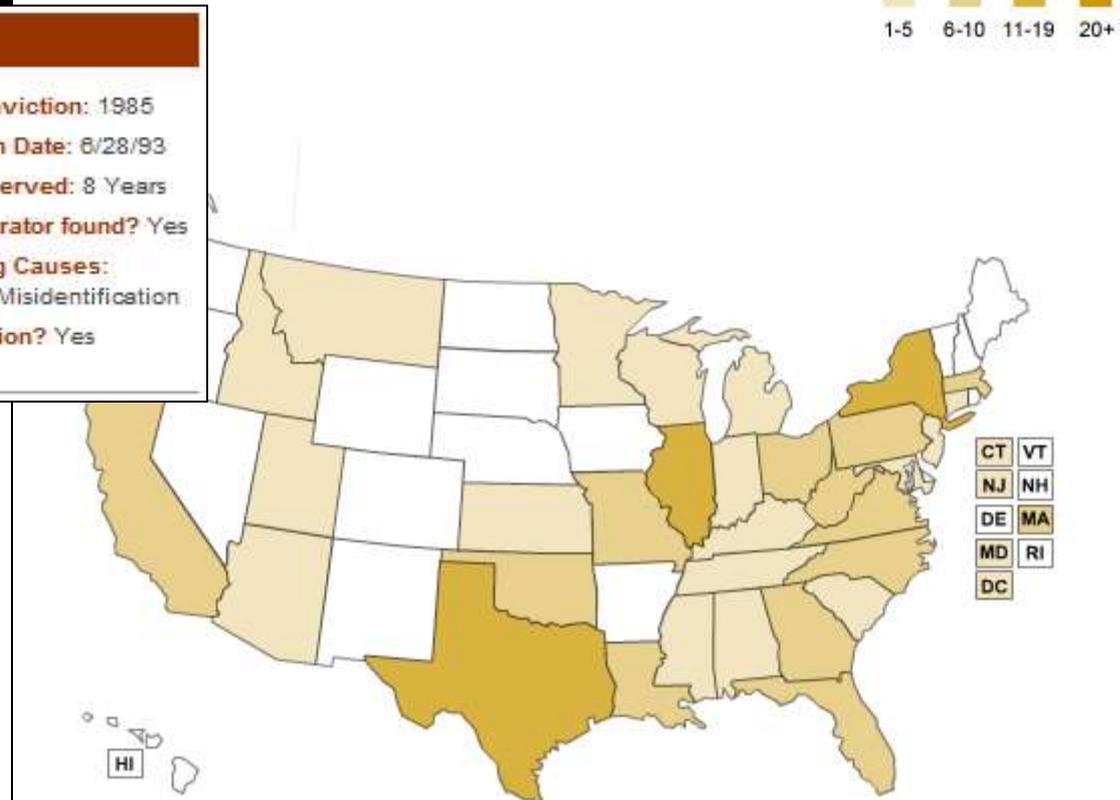
Rickey Johnson

Served 25 years in Louisiana for a crime he didn't commit.

Kirk Bloodsworth



Incident Year: 1984	Year of Conviction: 1985
Jurisdiction: MD	Exoneration Date: 6/28/93
Charge: Murder, Sexual Assault, Rape	Sentence Served: 8 Years
Conviction: First Degree Murder, Sexual Assault, Rape	Real perpetrator found? Yes
Sentence: Death	Contributing Causes: Eyewitness Misidentification
	Compensation? Yes

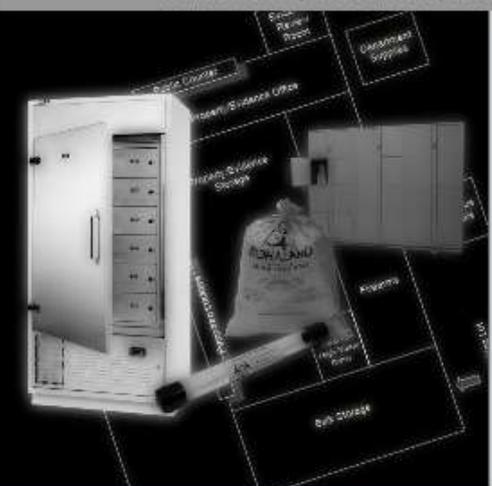
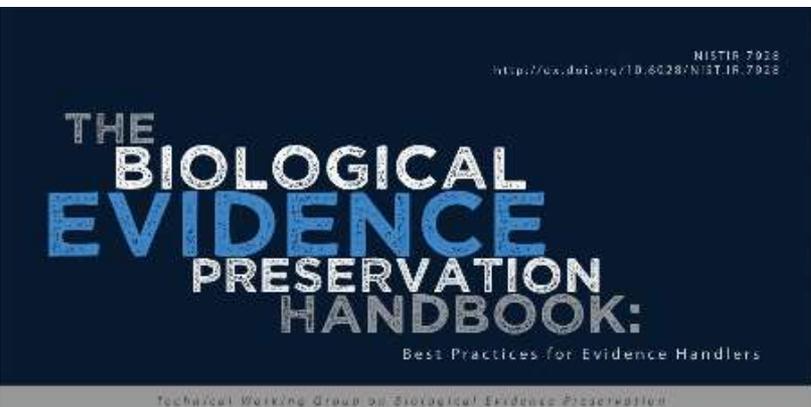


311 exonerated as of September 10, 2013

New Handbook on Biological Evidence Preservation

Available (as free pdf): <http://nvlpubs.nist.gov/nistpubs/ir/2013/NIST.IR.7928.pdf>

73 page handbook that makes recommendations for evidence retention, safe handling, packaging and storage, chain-of-custody and tracking, and appropriate disposal once evidence retention is no longer required by law



- Susan Ballou
- Phyllis S. Ba
- Larry Br
- Rebecca I
- Yvette Ba
- Dennis Das
- Lindsay Di
- Cynthia J
- Ralph Ke
- William
- Margaret
- Karen Lai
- Gerry La
- Joseph I
- Linda F. I
- Randy h
- Brian E. O
- Lisa Sch
- Stephanie
- Mark Stal
- Melissa T
- Shannan W

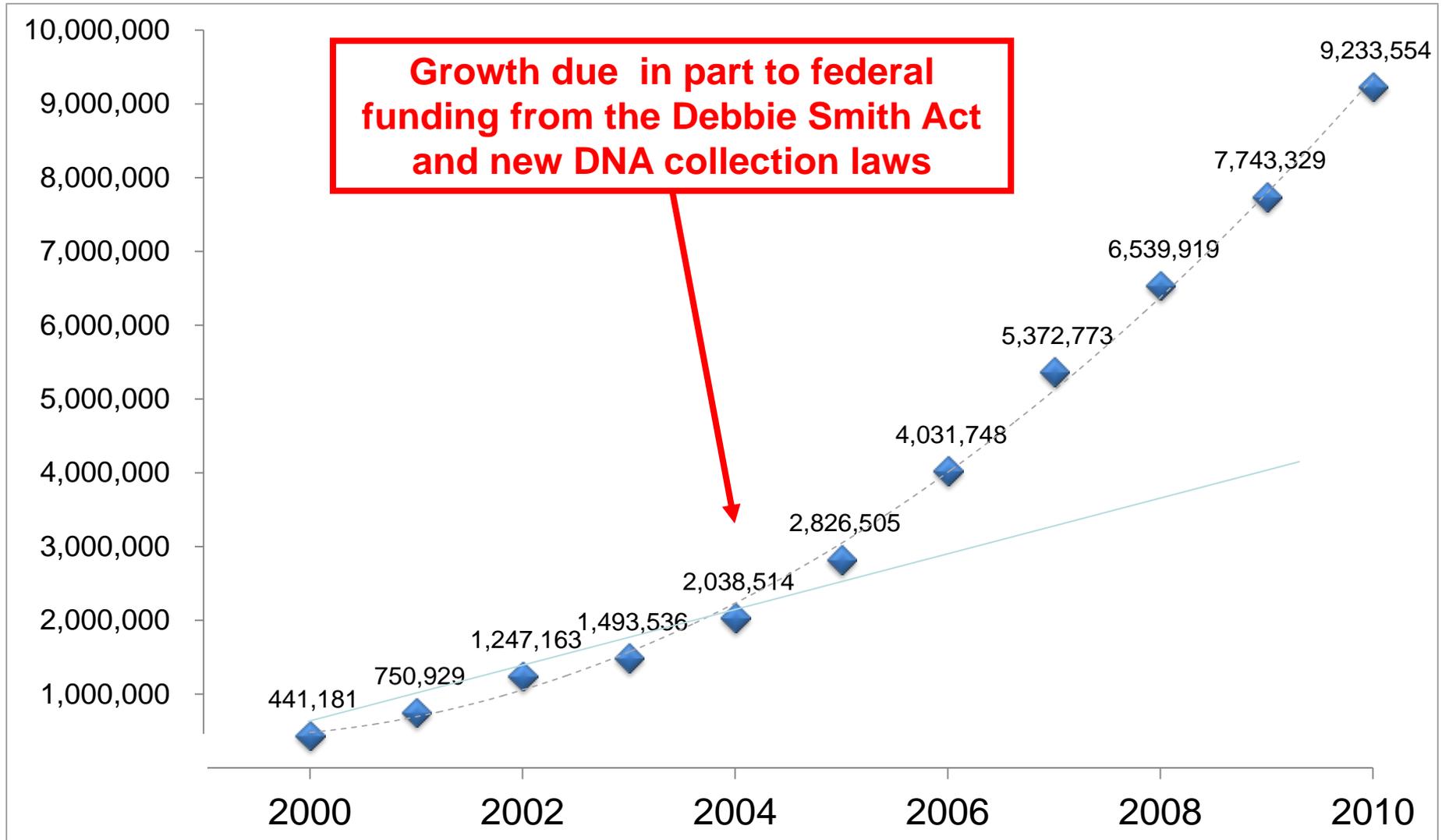
Table III-2: Long-Term Storage Conditions Matrix¹

Type of Evidence ²	Frozen	Refrigerated	Temperature Controlled	Room Temperature
Liquid Blood	Never	Best		
Urine	Best			
Dry Biological Stained Items			Best	
Bones			Best	
Hair			Best	Acceptable
Swabs with Biological Material			Best (dried)	
Vaginal Smears			Best	
Feces	Best			
Buccal Swabs			Best	
DNA Extracts	Best (liquid)	Acceptable (liquid)	Acceptable (dried)	



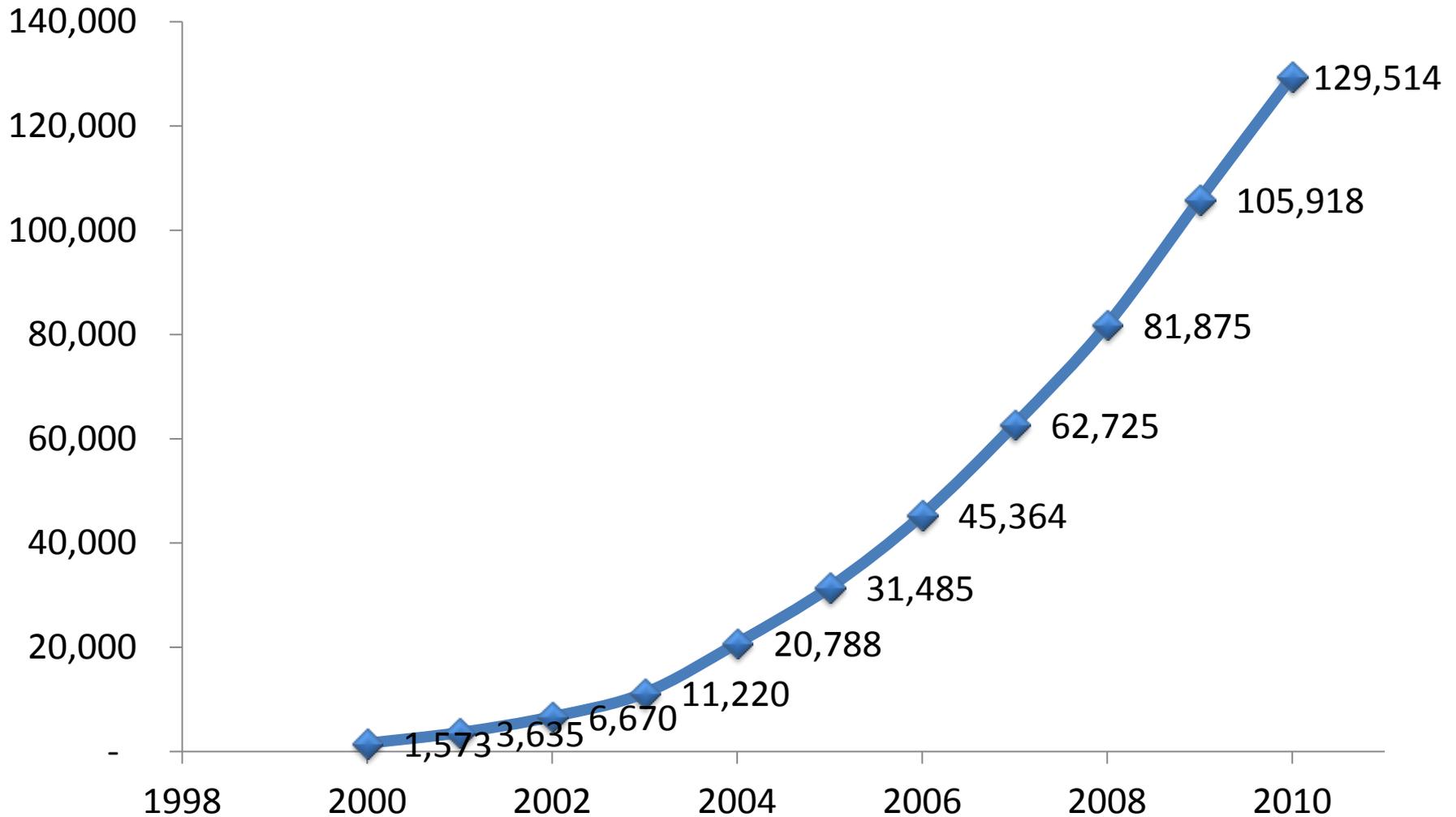
Released April 2013

Number of Offender DNA Profiles in the U.S. National DNA Database



Source: FBI Laboratory's CODIS Unit

Number of Investigations Aided in the U.S. National DNA Database



Source: FBI Laboratory's CODIS Unit

Growth of DNA Databases

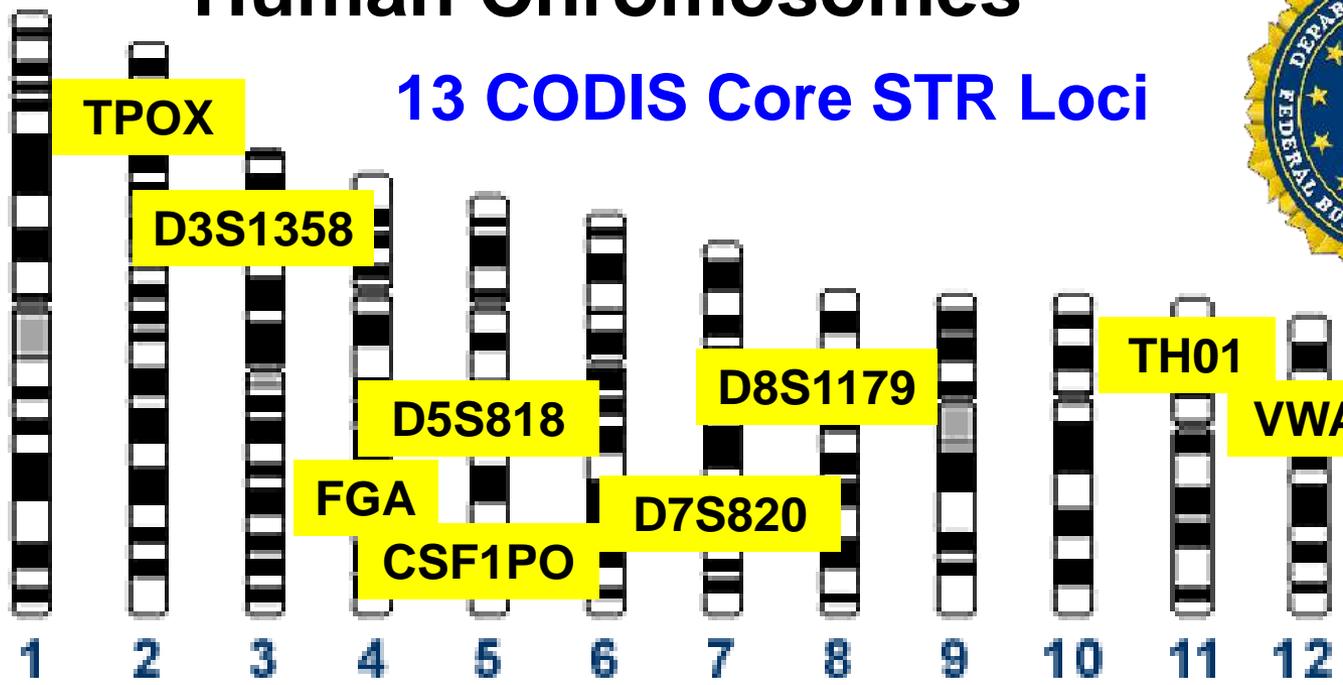
- Within the U.S., we have benefited from significant federal funding over the past decade
- Expanded laws now enable more offenders to be included (currently 26 states and federal government have laws to collect DNA from arrestees)
- Have effectively locked technology with core STR markers used to generate DNA profiles that now number greater than 10 million profiles

Core STR Loci for the United States

Position of Forensic STR Markers on Human Chromosomes

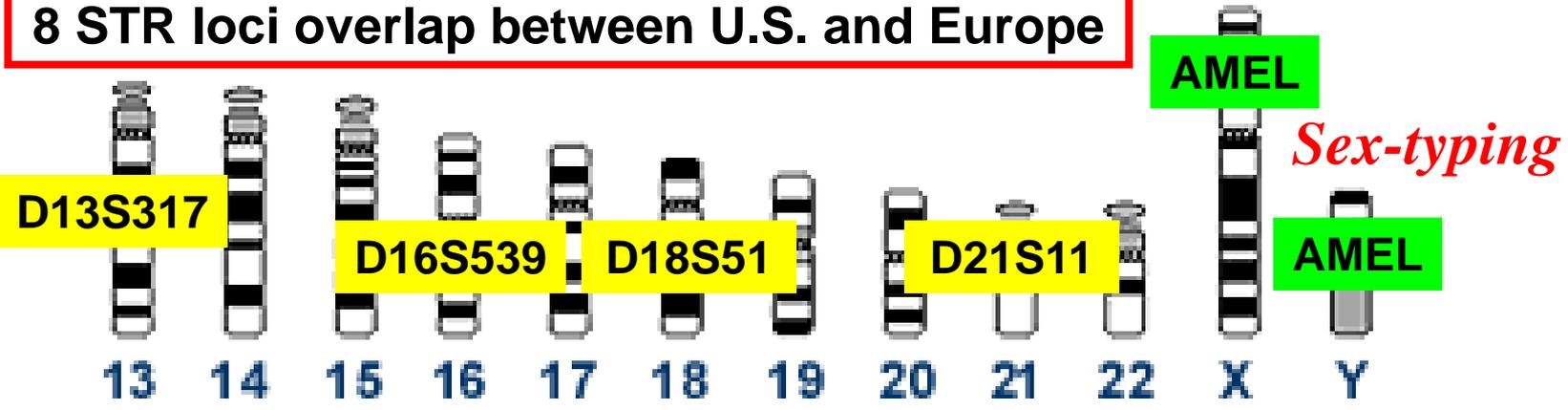


13 CODIS Core STR Loci



1997

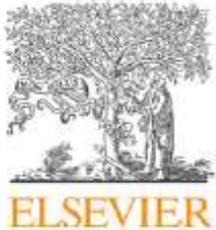
8 STR loci overlap between U.S. and Europe



Sex-typing

Expanding the U.S. CODIS Core Loci

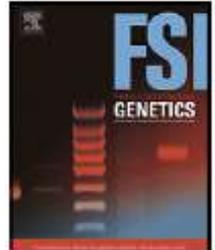
D.R. Hares (2012) Expanding the CODIS Core Loci in the United States. *Forensic Sci. Int. Genet.* 6(1): e52-e54
Addendum to expanding the CODIS core loci in the United States, *Forensic Sci. Int. Genet.* (2012) 6(5): e135



Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig



Letter to the Editor

Expanding the CODIS core loci in the United States

CODIS Core Loci Working Group

Formed in May 2010 to make recommendations
to FBI CODIS Unit

Douglas Hares (Chair) – FBI

John Butler – NIST

Cecelia Crouse – FL PBSO

Brad Jenkins – VA DFS

Ken Konzak – CA DOJ

Taylor Scott – IL SP

major reasons for expanding the CODIS core loci in the United States:

- (1) To reduce the likelihood of adventitious matches [7] as the number of profiles stored at NDIS continues to increase each year (expected to total over 10 million profiles by the time of this publication). There are no signs that this trend will slow down as States expand the coverage of their DNA database programs and increase laboratory efficiency and capacity.
- (2) To increase international compatibility to assist law enforcement data sharing efforts.
- (3) To increase discrimination power to aid missing persons cases.

Three major reasons for expanding the CODIS core loci in the United States

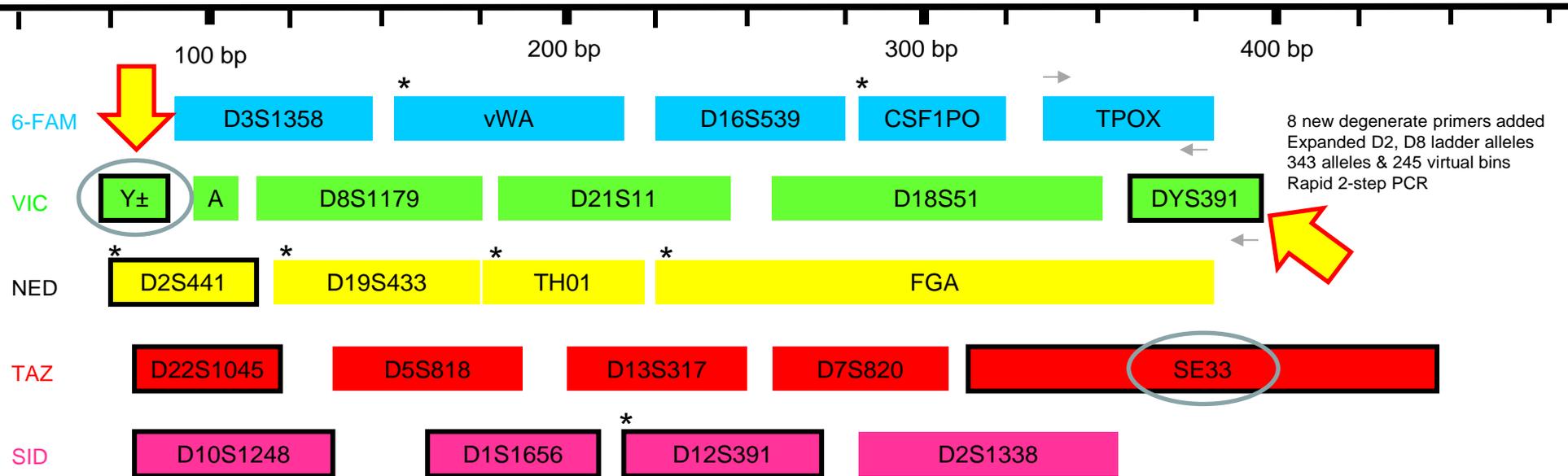
D.R. Hares (2012) *Forensic Sci. Int. Genet.* 6(1):e52-e54

- **To reduce the likelihood of adventitious matches** as the number of profiles stored at NDIS continues to increase each year
- **To increase international compatibility** to assist law enforcement data sharing efforts
- **To increase discrimination power to aid missing persons cases**

STR Kit Layouts by Dye Label and PCR Product Size

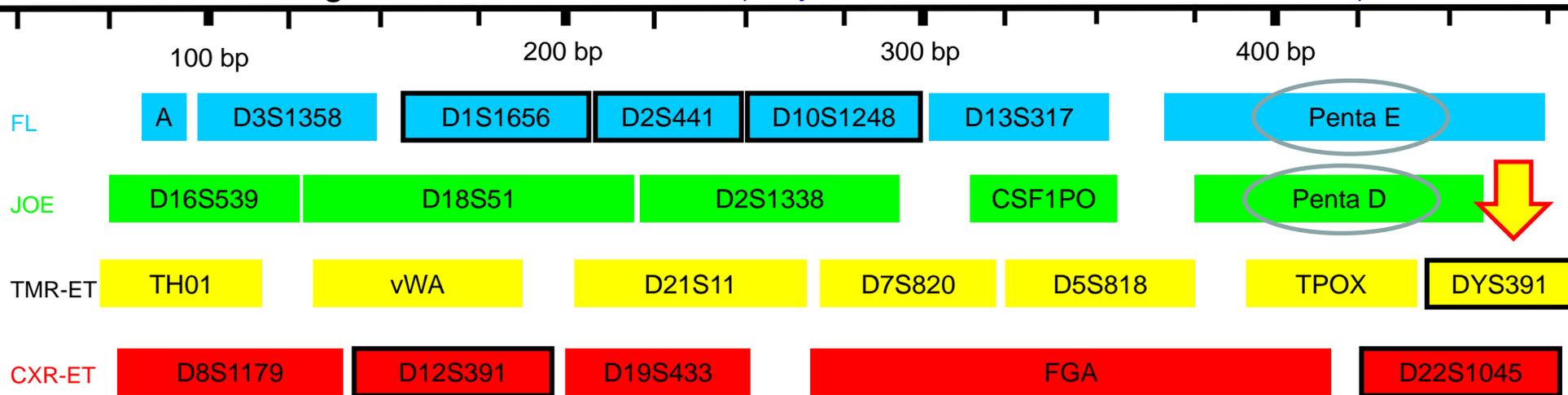
Life Technologies/Applied Biosystems **GlobalFiler** (6-dye – LIZ600 size standard)

24plex

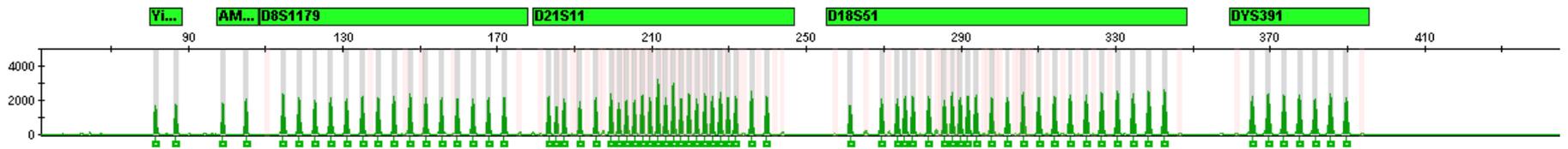
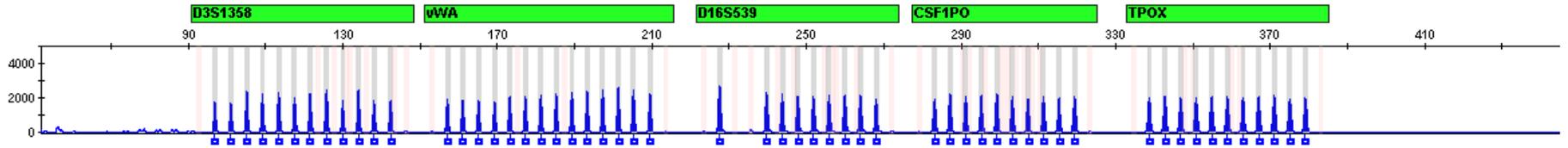


Promega PowerPlex **FUSION** (5-dye – CC5 internal lane standard 500)

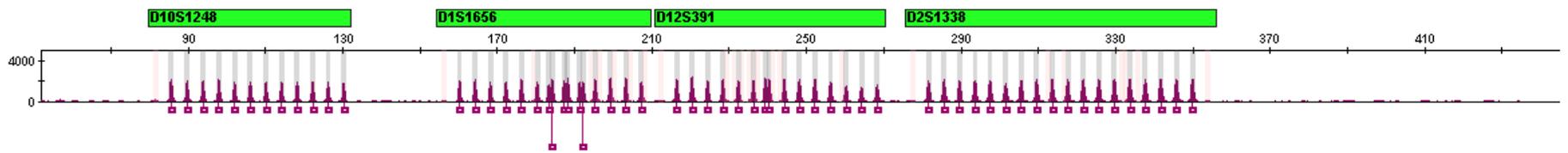
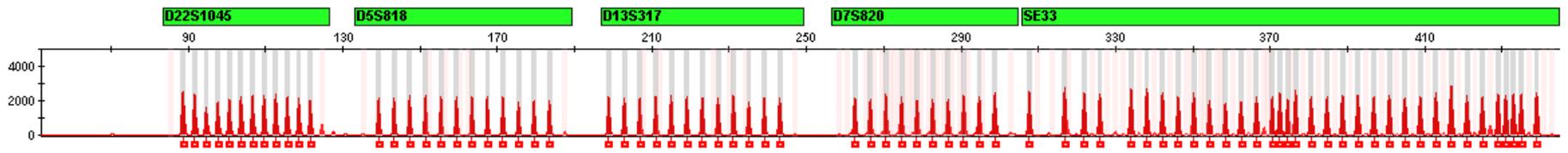
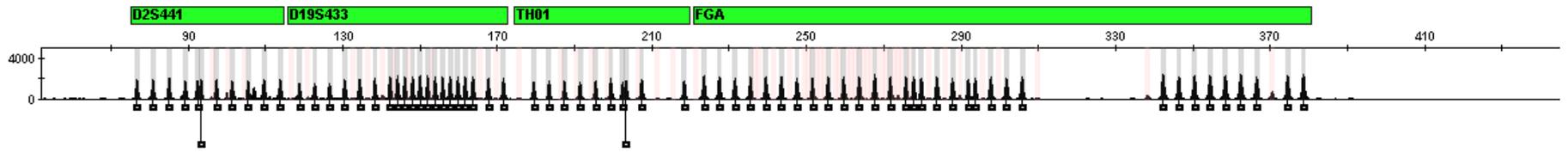
24plex



GlobalFiler Allelic Ladder



343 alleles across these 24 loci



Arrest Made in L.A. 'Grim Sleeper' Killings

Published July 07, 2010 | Associated Press



Print



Email



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Comments (0)



Recommend

42



Text Size



LOS ANGELES -- A one-time police mechanic was arrested and charged Wednesday in the serial killing of 10 people over 25 years after a DNA sample from his son was found to bear a close resemblance to DNA found on the victims.

Lonnie Franklin Jr., 57, was charged with 10 counts of murder, one count of attempted murder and special circumstance allegations of multiple murders that could make him eligible for the death penalty if convicted, District Attorney Steve Cooley said.



He is charged with 10 counts of murder and one count of attempted murder for a series of killings that date back to 1985.

Lonnie David Franklin Jr.

Putative Relative Is Found

- June 30, 2010: Second familial search of the California database yielded one likely relative
- Database profile belonged to Christopher Franklin (31 years old)
 - Profile added to the database in 2009 after a felony weapons possession charge
- Grim Sleeper profile matched C. Franklin's profile with one allele at all 15 loci
- Both individuals shared the same Y-STR profile, indicating a possible paternal relationship

Identifying the Grim Sleeper

- Given that the murders spanned at least 25 years, the paternal relationship was likely father-son
- Undercover police shadowed C. Franklin's father, Lonnie David Franklin, Jr., who lived in the vicinity of the murders
- Police collected a DNA sample from Lonnie Franklin
 - **Direct match between L. Franklin and the Grim Sleeper**

Rapid DNA Efforts



Pete Vallone Erica Butts

Accelerated Nuclear DNA Equipment
(ANDE) developed by **NetBio**



<http://ishinews.com/wp-content/uploads/2012/10/Rapid-DNA-Miles-1.58MB.pdf>

RapidHIT 200 developed by **IntegenX**



<http://integenx.com/wp-content/uploads/2010/06/RapidHIT-200.png>

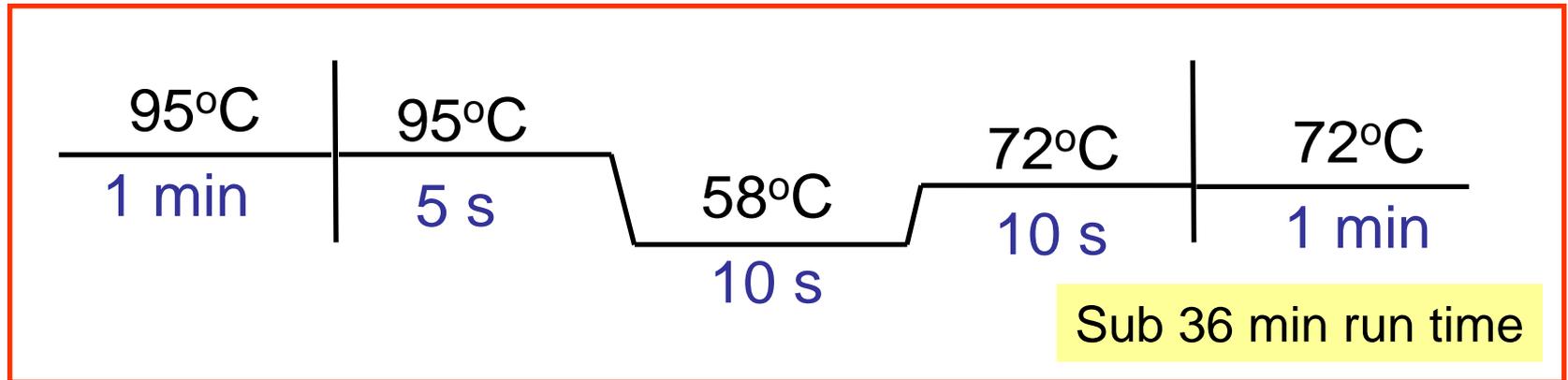
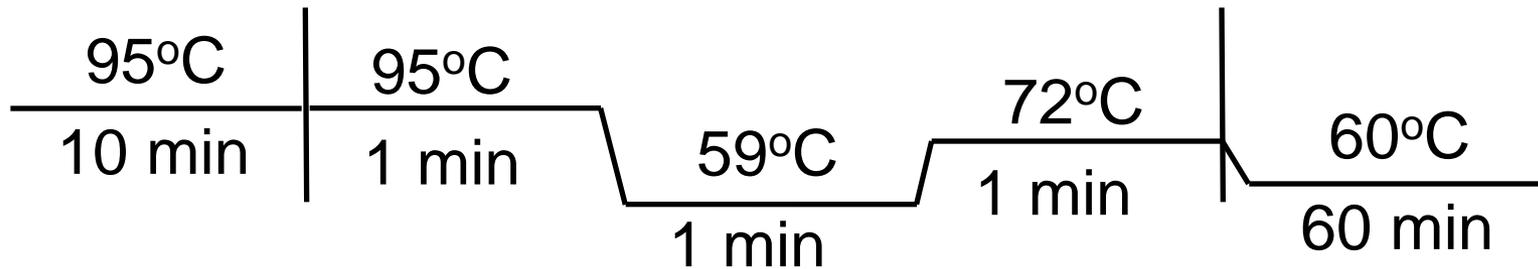
- Evaluating ANDE (NetBio) and IntegenX rapid DNA instruments
 - both instruments are capable of swab in → STR profile out in less than 90 minutes without user intervention
- Exploring rapid DNA techniques including direct PCR and rapid PCR
 - STR profiles generated in <2 hours with standard lab equipment and rapid protocols
 - See ISHI 2012 poster available on STRBase “Rapid DNA Testing Approaches for Reference Samples”

**Fastest results swab-to-profile
(Identifiler): 57 minutes**

Rapid PCR Thermal Cycling Profile

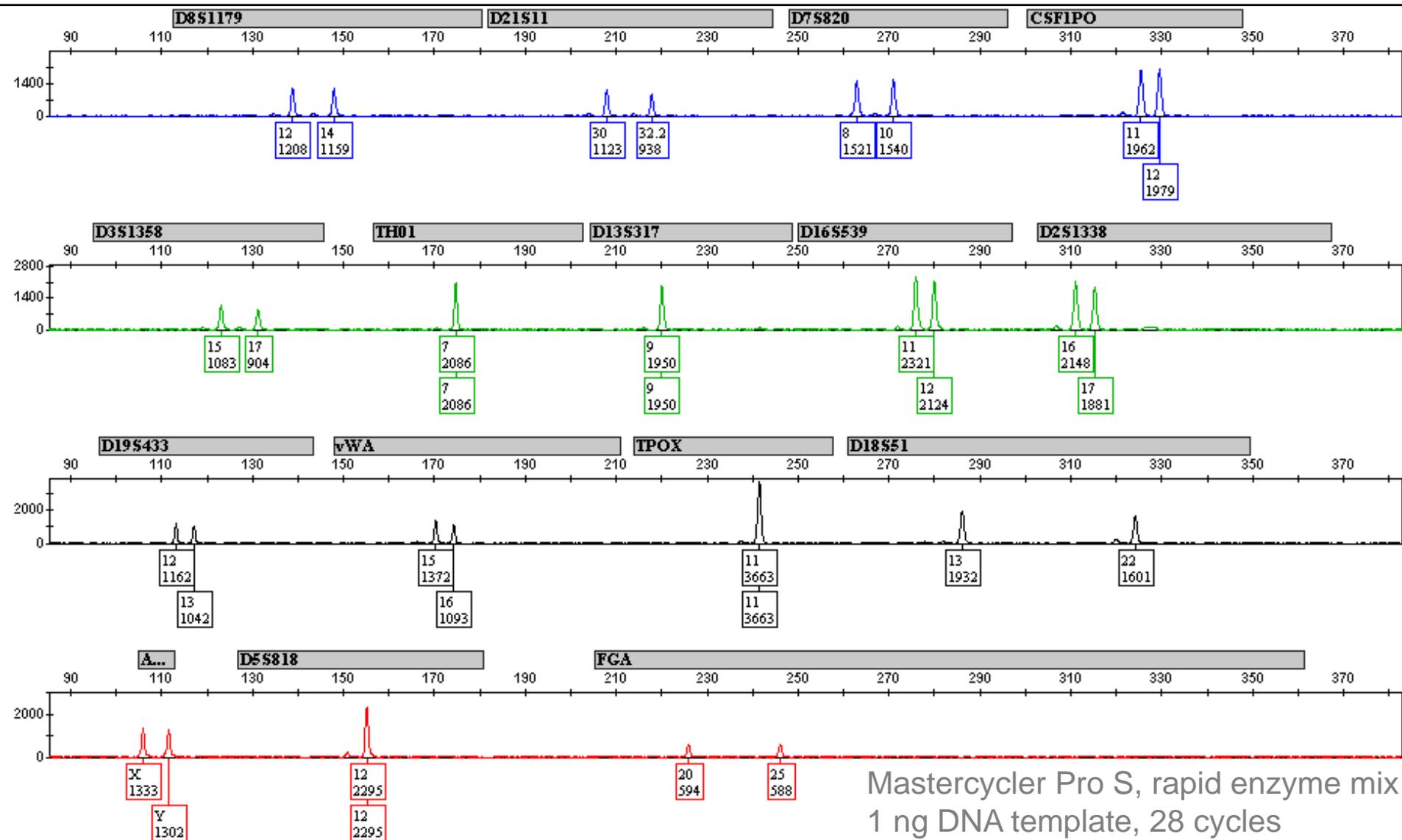
Identifiler STR kit

28 cycles of PCR



Maximum heating/cooling rate of ~2 to 6°C/s (cycler dependent)

Full Identifiler STR Profile with 19 min PCR



Mastercycler Pro S, rapid enzyme mix
1 ng DNA template, 28 cycles

Potential Applications with Rapid PCR Capabilities

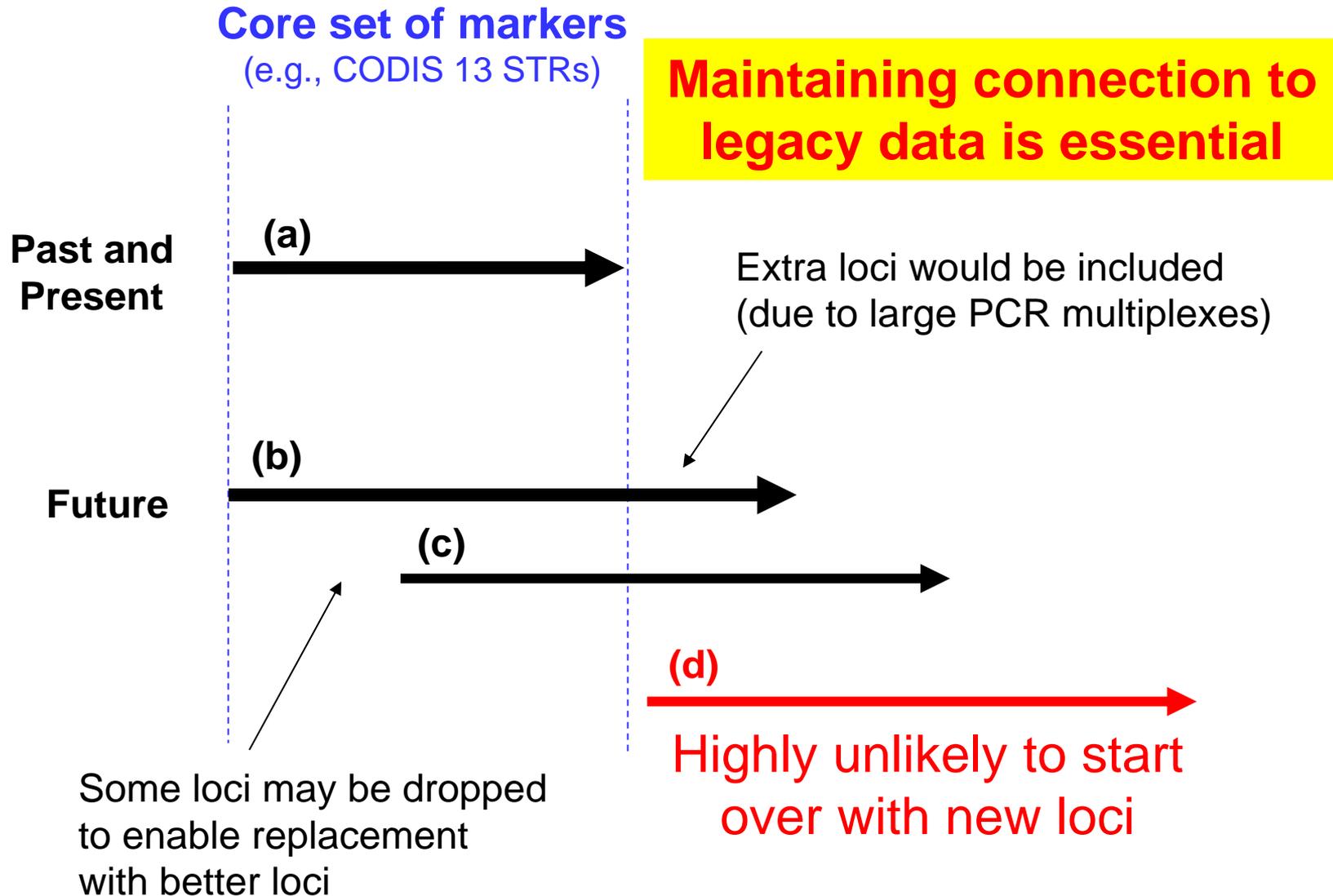
- **Improve overall laboratory throughput**
 - Multiplex PCR amplification is already in many situations the longest part of the DNA analysis process (depending on DNA extraction and DNA quantitation methods)
 - With increased use of robotic sample preparation and expert system data analysis, bottleneck for sample processing will shift to time for PCR amplification...
- **Enable new potential DNA biometric applications**
(because the overall DNA analysis process is faster)
 - Permit analysis of individuals at a point of interest such as an embassy, an airport, or a country border

A “Crystal Ball” to the Future?

<http://medicalcenter.osu.edu/images/healthconnections/winter2003/dnaCrystalBallIllustration.jpg>



Possible scenarios for extending sets of genetic markers to be used in national DNA databases



STRs vs SNPs Article

Butler *et al.* (2007) STRs vs SNPs: thoughts on the future of forensic DNA testing. *Forensic Science, Medicine and Pathology* 3:200-205.

Forensic Sci Med Pathol (2007) 3:200–205

DOI 10.1007/s12024-007-0018-1

ORIGINAL PAPER

STRs vs. SNPs: thoughts on the future of forensic DNA testing

John M. Butler · Michael D. Coble ·

Peter M. Vallone

- **SNPs are unlikely to replace STRs** for routine forensic DNA testing due to challenges with high-level multiplexing and mixture detection/interpretation
- Most likely use of SNPs will be as ancestry-informative markers (AIMs) **for sample ethnicity estimation**

Geographical Origin Prediction



- Lao O, van Duijn K, et al. (2006) **Proportioning whole-genome single-nucleotide-polymorphism diversity for the identification of geographic population structure and genetic ancestry.** Am J Hum Genet 78: 680-90.
- Phillips, C., Salas, A., et al. (2007) **Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs.** FSI: Genetics 1: 273-280.
- Halder, I., Shriver, M., et al. (2008) **A Panel of Ancestry Informative Markers for Estimating Individual Biogeographical Ancestry and Admixture From Four Continents: Utility and Applications.** Hum Mut 29: 648-658.
- Pereira R., Phillips C., et al. (2012) **Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing.** PLoS One;7(1):e29684.

Phenotypic Trait Prediction

Traits of interest

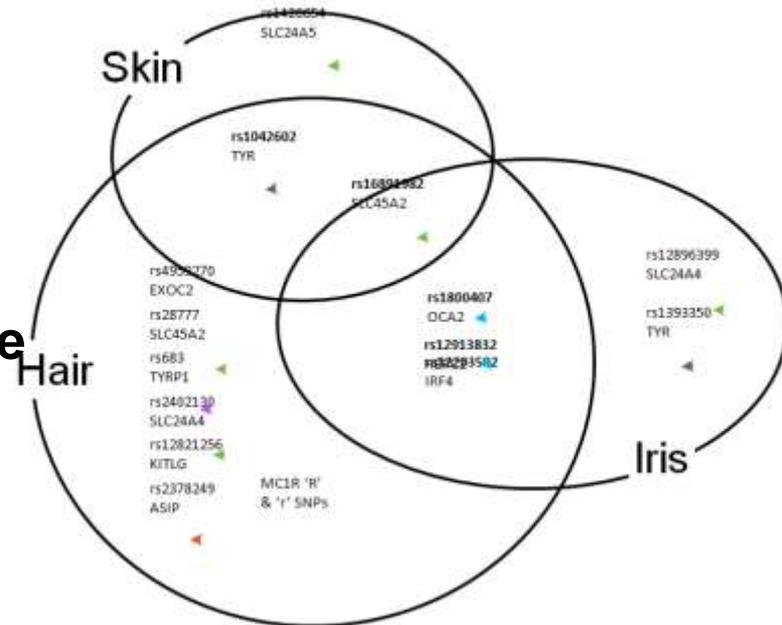
- Traits whose variation may be classified on discreet categories.
- Regulated by a relatively low number of genes.
- Fine example: Iris and hair pigmentation.



Blue

Intermediate

Brown



Next Generation Sequencing

Forensic Applications

- Going in depth **into** STR loci and beyond
 - STRs are useful for legacy (databases)
 - SNPs within STRs identify ‘sub-alleles’
 - Millions of bases of sequence variants (SNPs)
- Opens up new human identity applications: biogeographical ancestry, externally visible traits, complex kinship, **degraded samples, mixtures, other applications**

Applications are currently being addressed
by the forensic genetics community (*Kayser and deKnijff 2011*)

Next Generation Sequencing Workshop

- Interagency Workshop on the use of Next-Generation DNA Sequencing for Human Identification and Characterization (Jan 31 2012)
- Discussion of forensic applications of NGS (NIST, DoD, FBI, DHS) – materials can be found at:
 - http://www.nist.gov/mml/bmd/genetics/ngs_hid_workshop.cfm
- We are in the process of looking at platforms to characterize forensic markers (mitochondrial, STRs, SNPs)
- Evaluate accuracy, reproducibility, identify initial requirements for a NGS forensic reference material

Some Thoughts on the Future...

- **PCR amplification**
 - Faster enzymes to enable rapid PCR
 - More robust enzymes and master mixes to overcome inhibition
- **Instrumentation**
 - More dye colors to aid higher levels of multiplexing
 - Rapid, integrated devices
 - Alternatives to capillary electrophoresis: ~~PLEX-ID~~ and NGS
- **Quantitative information**
 - qPCR and digital PCR
- **Marker systems**
 - Expanding sets of STR loci for growing DNA databases
 - Other marker systems: SNPs, InDels, X-STRs, RM Y-STRs
 - Body fluid identification with mRNA, miRNA, and DNA methylation
 - Phenotyping for external visible characteristics
 - Challenges with potential whole genome information
- **Data interpretation**
 - Probabilistic genotyping for low-level DNA and mixture interpretation
 - Probability of dropout

We Need Continued Efforts to Improve DNA Interpretation (especially low-level DNA and mixtures)

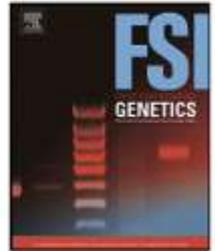
Forensic Science International: Genetics 6 (2012) 677–678



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Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig



Editorial

Focus issue—Analysis and biostatistical interpretation of complex and low template DNA samples

December 2012 – Forensic Science International: Genetics, volume 6, issue 6

Forensic Science International: Genetics 6 (2012) 679–688



Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig



DNA commission of the International Society of Forensic Genetics:
Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

P. Gill^{a,b,*}, L. Gusmão^c, H. Haned^d, W.R. Mayr^e, N. Morling^f, W. Parson^g, L. Prieto^h,
M. Prinzⁱ, H. Schneider^j, P.M. Schneider^k, B.S. Weir^l

DNA Mixture Interpretation

April 12, 2013 Webcast



NIST FORENSIC
SCIENCES

<http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation.cfm>

- **8-hours of DNA mixture interpretation training**
- **11 presentations from five different presenters**
 - John Butler, Mike Coble, Robin Cotton, Bruce Heidebrecht, Charlotte Word
- **20 poll questions** asked via SurveyMonkey (>600 participated)
 - Addressed additional questions sent via email or Twitter
- **>1000 participants** (almost entire U.S. represented and >10 countries)
- **Available for viewing or download** for at least six months (storage costs may limit longer-term storage)



Left to right:

Gladys Arrisueno (NIST, Twitter feed monitor & poll questions)

John Paul Jones (NIST, webcast organizer)

Mike Coble (NIST, presenter)

John Butler (NIST, presenter & organizer)

Charlotte Word (Consultant, presenter)

Robin Cotton (Boston University, presenter)

Bruce Heidebrecht (Maryland State Police Lab, presenter)

DNA Interpretation Training Workshops

melbourne

25th World Congress of the International
Society for Forensic Genetics
2 – 6 September 2013

September 2-3, 2013

Two days of basic and advanced workshops
on DNA evidence interpretation



Handouts and reference list available at

<http://www.cstl.nist.gov/strbase/training/ISFG2013workshops.htm>

The Workshop Instructors



**Mike Coble
(NIST)**

**Peter Gill
(U. Oslo)**

**Jo Bright
(ESR)**

**John Buckleton
(ESR)**

**Duncan Taylor
(FSSA)**

**John Butler
(NIST)**

The Future of Forensic DNA

is Similar to the Olympic Motto of
“Swifter, Higher, Stronger”



Resources

Training

Action

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Thank you for your attention

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NIST publications and presentations are available at:
<http://www.cstl.nist.gov/strbase/NISTpub.htm>